Taq-&LOAD™ Mastermix 5xC

Cat. # 11EPTAL100
1 ml

Storage : -20°C

For research use only. Not for use in diagnostic procedures

Mix thoroughly before the first use.

Avoid successive freeze thaw cycles. Product is stable 6 months at +4°C

Taq-&LOAD™ (5xC) is a **Ready to Use Mastermix** containing all the components required for performing PCR (recombinant *Taq* DNA Pol, dNTPs, PCR buffer, $MgCl_2$) and also a densifying agent and a Red Purple dye for Direct Loading of the PCR product on agarose gels.

Simply add 1 volume of Taq-&LOAD™ (5 x C) Mastermix to 4 volumes of water containing DNA template and specific primers. After PCR cycling, directly load your amplification reaction onto agarose gel. The red purple dye migrates at 400 bp on a 1.2% agarose gel.

Final concentrations of 1xC Mastermix are: 1.5 mM $MgCl_2$, 200 µM dNTPs
The use of a ready-to-use mastermix ensures reproductibility of results for sets of repeated PCR assays. By avoiding multiple pipetting, the risk of contamination and volume errors are greatly reduced.

Taq-&LOAD can be stored for 6 months at 4°C and thus avoids time waste in thawing the reaction components

A. Unit definition:
One unit of DNA polymerase is the amount of enzyme required to catalyse the incorporation of 10 nanomoles of nucleosides into a DE81 adsorbable product within 30 min at 74°C under assay conditions.

B. Absence of contamination:
The *Taq* DNA Polymerase used is a highly purified recombinant enzyme, free of any nickase, endonuclease, 3'exonuclease and ribonuclease.
All other components used in Taq-&LOAD™ (5xC) Mastermix, are checked to be free of any endo or exonuclease.

C. PCR performances assay:

1. A 400 bp region of the human β-globin gene was amplified using specific primers (25 pmol each) in a 50 µl final volume reaction and 10 ng of genomic DNA from human blood cells. PCR products were compared to the products obtained from traditional PCR reaction with 1U of *Taq* DNA Polymerase (Cat# EPTQA025) and 200 µM dNTP. The PCR profiles and the yields of PCR products are equivalent.

2. A 400 bp region was amplified from 1fg to 50 pg of pBR 322 plasmid.
PCR program used: Initial denaturation 4' at 93°C.
Then 30 cycles of (30'' at 91°C - 30'' at 55°C - 1' at 70°C) and a final extention of 10' at 70°C.

3. Amplification of a 4.0 kb fragment from 10 ng of genomic DNA as a template, systematically generates a reproductible quantity of PCR product.
PCR program used: Initial denaturation 5' at 93°C.
Followed by 25 cycles of (30'' at 94°C - 2' at 62°C - 5' 30'' at 72°C) and a final extention 10' at 72°C.

D. Reaction conditions:
Taq-&LOAD™ (5xC) Mastermix is optimised for efficient and reproducible standard PCR. Nevertheless, each type of amplification needs an individual optimisation and depends on the type of template, the complementary sequences of the primers and the length of the amplified product. The following informations are provided as general advice:

All reagents are added in sterile conditions, in a PCR microtube, on ice.
Reaction volume..... 50µl
Taq-&LOAD MasterMix 5xC.....10µl
Additional $MgCl_2$.....up to 5 mM
Each primer.....10 to 50 pmoles
DNA template.....10 pg to 100 ng (according its origin)
Reaction volumes can be scaled down or up, if desired. Take care than you have 1U of *Taq* DNA Polymerase in a final reaction volume of 50µl.
For Suggested PCR Programme, please see point **G.**

After PCR, 5-10 µl of reaction can directly be loaded onto an agarose gel and visualized by ethidium bromide staining. Red dye migrates at 400 bp in a 1.2% agarose gel.

E. Downstream applications:
- PCR products can directly be re-amplified by "nested PCR".
- The Taq-&LOAD components do not interfere with restriction enzyme digestions when up to 50 % of the PCR volume are used.
- **Taq-&LOAD™** (5xC) is compatible with RT-PCR experiments.
- *Taq* DNA polymerase adds overhang A on PCR product thus T/A cloning could be performed.
- If necessary, Red dye can be removed by ethanolic precipitations or by GENECLEAN turbo PCR cleaning kit.

F. Guidelines for primer sequence design:

- Primers should have at least a minimal complementary sequence of 16 nucleotides to the template, for amplification on genomic DNAs of human and animals and 21-22 nucleotides for plants. That length should not exceed 28-30 bases.
- Addition of several mismatched bases (to introduce a restriction site) might occur at the 5' extremity of the primer
- A single mismatched base (introduction of a point mutation) can be located within or at any extremity of the primer sequence
- Addition of more than one mismatched base at the 3' end of the complementary primer will provoke a "breathing" effect preventing amplification
- Final base composition should always be 50-60% (G+C)
- Tm (melting temperature) of both primers should be equivalent and not exceed 70°C. A simple and rapid formula to calculate the Tm is (4°C per G/C + 2°C per A/T).
- The annealing temperature (Ta) preferably about 5°C below the lowest Tm of the pair of primers to be used.
- 3' ends of primers should not be complementary to avoid primer dimers
- Primers with secondary structures can be shortened at either 5' or 3' extremities to make the hairpin unstable
- In all cases, when the hairpin cannot be avoided, the Tm of the hairpin must be significantly lower than the final annealing temperature, to enable denaturation of the secondary structure during the PCR process.

G. PCR program:

1. An initial denaturation step of 3-5 min at 93-95°C is recommended to ensure complete separation of the two DNA strands.

2. For subsequent cycles, a denaturation time of 15-60 sec at 93-95°C is sufficient for PCR fragments < 3-4 kb.

As high temperatures (like 95°C) decrease the pH of reaction and thus, favours depurination, the time of denaturation must be reduced to 20 sec to ensure the amplification.

3. The annealing temperature depends directly on the length and the Tm of the primers. A simple formula to calculate the Tm is 4°C per G/C and 2°C per A/T. The annealing temperature should be placed 5°C below.

One consequence of too low a Ta, is a reduced yield product and appearance of non-specific amplicons.

A consequence of too high a Ta, is a lower yield of product, as the likelihood of primer annealing is reduced and the initial template may not remain denatured.

4. For primers lengthened with non-matched bases or containing point mutations, define the annealing temperature of the sequence complementary to the original template and use it for the first 3 to 5 PCR cycles. To define the annealing temperature for the last 20 or 30 cycles, consider the total sequence of the primers that will hybridise to the newly amplified fragments.

5. Most primers will anneal efficiently in 30 to 60 sec or less, unless Ta is too close to Tm, or primers are unusually long.

6. For the elongation step at 72°C, 1 min per 1 kb is sufficient when amplifiable DNA is over 2 kb. Under 2 kb, this rule does not hold. Check in the user guide of your thermocycler, as the time needed for a specific length might change with the machine.

7. The number of cycles depends on the starting concentration of the target DNA. Between 20 to 37 cycles are recommended. Above 37 cycles, problems of specificity due to depletion of PCR components may occur. The PCR reaction is then subject to an attenuation in exponential rate of product accumulation in late stages of a PCR.

8. At the end of the PCR program, it may be desirable to perform a final extension for up to 10 min at 72°C. This reduces the number of incompletely amplified fragments.

H. Troubleshooting:

1. Little or no amplification observed:

- Verify the primers sequence and the absence of secondary structures, redesing the primers.
- Check the primers Tm, and decrease the annealing temperature by 2 to 5°C.
- Check the template quality or the presence of inhibitory factors. Repurify the DNA.
- Check the GC content of template. DMSO or Betaine could be useful for amplifying GC rich regions of DNA.
- Raise initial denaturation temperature or increase its time.
- Increase the elongation time.
- Increase the number of cycles by 3-5 (no more than 40 total cycles).
- Vary the quantity of the template, the primers.
- Adjust $MgCl_2$ concentration.

2. Multiple bands or smearing observed:

- Check the Tm of the primers and their homologous sequences to the template.
- Verify the primers sequence and the absence of secondary structures.
- Increase the hybridisation temperature of the primers by 2°C to 5°C to avoid non-specific hybridisation. If too small amounts of specific product are obtained, use a "touch-down" program (i.e. start the PCR with few cycles at a Ta higher than both primers Tm. Then reduce the Ta for each cycle to 5°C under the initial Tm).
- For primers lengthened with non-matched bases or containing point mutations, check that the chosen hybridisation temperature is adapted
- Lengthen the homologous sequence of the primer to the template.
- When mismatched bases are added, increase the annealing temperature of the homologous sequences of the primers by 2°C to avoid non-specific hybridisation of the non-homologous sequences of the primers. And/or increase the annealing temperature of the complete primer by 2°C for the last 20-30 cycles.
- Reduce the number of cycles.
- Reduce the concentration of DNA template, of primers.

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